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Study on the immunopathologic effects of 4(3H)-quinazolinone-2-ethyl-2-phenyl ethyl (QEPE) in newborn Balb/C mice

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Abstract

Quinazolinones are heterocyclic and water insoluble compounds with various pharmacological and biological characteristics (antibacterial, antiswelling, antifungal, parkinson and etc.). They are used for treatment HIV and cancer. This study investigated the effects of 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) as a new quinazolinons compounds on the spleen and immunocompetent cells of newborn Balb/C mice. Pregnant mice were divided into 3 groups (n=10) of control, sham and experimental, received distilled water, methyl cellulose %0.05 (the solvent) and 100 mg/kg body weight (ip) of QEPE (most effective dose), respectively, on days 8 to 15 of gestation. Examinations indicated an increase in weight of spleen in experimental group. Pathological studies showed increase in capsule thickness and number of macrophage cells of experimental group. Statistical analysis showed significant differences in morphological studies between experimental, sham and control groups. The statistical data on capsule thickness and number of macrophage cells indicated significant differences between groups. Detailed observations showed an increase in the volume of monocyte, neutrophile and eosinophile, in response to QEPE, but the volume of lymphocyte and basophile were similar in experimental, control and sham groups. The damages caused by this dose of QEPE could have been the reason for the increase in the number of immonucompetent and macrophage cells. Some studies showed damages to the organs such as livers and hearts would lead to the increase in the thickness of spleen capsule, consequently, increase in its weight and leading to splenomegaly. So, QEPE can not be an appropriate candidate for drug development. QEPE in lower doses might be an appropriate candidate for increase of immune system resistance.

Key Words: Quinazolinones, Immunocompetent cells.

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Introduction

Quinazolinones are heterocyclic and water insoluble compounds (David et al., 2005), with various pharmacological; antimicrobial, antifungal, antitumor, anticonvulsant, antiinflammatory, antiallergy, antimalaria (James et al., 1990, Pines et al., 2000, Buyuktimkin et al., 2006, Jatav et al., 2006, Yesilada et al., 2004, Ouyang et al, 2006 and Baek et al. 2004). They are more efficient than other chemicals in inhibiting HIV and cancer. They inhibit HIV-1 reverse transcriptase and use for treatment of HIV (Corbett et al., 2000 and Boumendjel et al., 2005).

The mechanism of the effects of quinazolinones on the embryonic cells is not clear yet, but there are quite a few reports showing its toxic characteristics. They inhibit polymerization of tubulin (Mann et al., 2006) and pass through placental barriers (Perretti and Zilletti, 1969), so there is a possibility that it has some sort of toxic and teratogenic effects on embryos. Pervious studies at the department of zoology, faculty of biological science, university of Shahid-Beheshti, 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) can causes morphological, skeletal and histological abnormalities in Balb/C mice embryos (Shams lahijani et al., 2006, Shams lahijani and Aounegh, 2007, Etemad and Shams lahijani, 2007, Fadavi and Shams lahijani, 2007, Rajabi and Shams lahijani, 2007).

In this regard spleen is involved in removed of pathogenic agents and some external components by phagositic cells (macrophage cells) , and some experiments have shown that , alfatoxin , 2-metoxyacetic , 2-metoxyethanol , 2-etoxyethanol , deoxynivalenol , dioxins , ethanol , parachloronitro-benzene and malaria toxins can cause damages and abnormalities in the spleen (Jimenez et al., 2005, Sehu et al., 2007, Riddle et al., 1996, Matsumoto et al., 2006, Sama and Moro, 1993, Paula et al., 1998 and Bordmann et al., 1997). Immunocompetence is vital in maintaining the overall health of an organism and is extremely sensitive to pathogens and toxins (Institoris et al., 2001). Measurement of spleen weight and immunocompetent cells allow evaluation immunopathologic condition of some animals in response to chemical exposure (Institoris et al., 2001, Keith, 2002 and Jennifer et al., 2004). We interested that whether treatments pregnant mice with QEPE would affect the spleen morphology and histology and immunocompetent cells of Balb/C mouse fetuses. Corbett et al. (2000) showed that some compounds of quinazolinone are very important in treatment of HIV by inhibiting of reverse transcriptase and increasing of immuno system resitance. So, another purpose of this study is, whether this compound is useful for immuno syetem resistance.

Materials and methods

Balb/C mice were housed in 24±1° C ,65 ± 0.5% humidity and lighted controlled room (12h light-dark), provided with lab chow (pellets) and tap water. They were originally obtained from Razi Institute (Tehran, Iran); Random breedings were implemented in our local facility, animal room, with breeding, operation and maintenance sections. Males mated virgin females at overnight, observing vaginal plugs presented day zero of pregnancy.

The new derivative of qunazolinones: 4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE), synthesized at Department of Chemistry, Faculty of Science, University of Shahid- Beheshti, Tehran, Iran (Dabiri et al., 2004) were used for IP injection. So, pregnant mice were divided into 3 groups (n=10) of control, sham, and experimental, received distilled water (10ml/kg), methyl cellulose %0.05 (10ml/kg) (the solvent of quinazolinones) and 100 mg/kg Balb/C body weight of QEPE (most effective dose), respectively, by IP injection, on days 8th to 15th of gestation. 5day old newborns were killed by cervical dislocation. The spleen was excised from each mouse and measured in weight. Then they were fixed in formalin %10, stained with H&E (Hematoxilin & Eosine) for

histological and pathological studies under compound microscope. For counting of immunocompetent cells, blood samples from the heart were collected by using heparinized tubes. Counting was carried out manually by Neubaur chamber and using Turky's solution (Brecher et al., 1992). Data were analyzed with statistical packages for social sciences (SPSS,version 12.0).Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed using One-Way ANOVA. Level of significance difference was P<0.05.

Table 1: Effects of 100 mg/kg of QPPE on the spleen of newborn Balb/C mice.

Parameters	Control	Sham	Experimental
Spleen Weight	0.04178 ± 0.0002	0.04185 ± 0.0001	0.04557 ± 0.0005*
Macrophage Cells	159.03 ± 2.53	158.8 ± 1.56	490.93 ± 2.37*
Capsule Thickness (μm)	2.98 ± 0.061	2.98 ± 0.03	4.65 ± 0.037*

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control and sham groups

Table 2: Effects of 100 mg/kg of QEPE on the immunocompetent cells of newborn Balb/C mice.

Parameters	Control	Sham	Experimental
Lymphocyte	67.47 ± 0.184	67.48 ± 0.189	67.49 ± 0.088
Monocyte	2.35 ± 0.022	2.36 ± 0.013	2.84 ± 0.017*
Neutrophile	29.64 ± 0.049	29.64 ± 0.046	30.10 ± 0.046*
Eosinophile	2.45 ± 0.012	2.44 ± 0.012	2.56 ± 0.008*
Basophile	0.36 ± 0.0057	0.36 ± 0.0078	0.36 ± 0.0061

Values are mean ± SEM (n=10); *P < 0.05 (significantly different) vs. control and sham groups.

Results

All investigated spleens and immunocompetent cells data are recorded in table 1 and 2. Treatment of mice with 100mg/kg of QPPE can increase weight of spleens (Figs 1-3). There was highly significant increase in spleen weight in experimental group compared the control and sham groups. Treatment of mice with 100mg/kg QPPE has increased the number of macrophage cells and capsule thickness in spleens (Figs 4-7). There were not significant differences between control and sham groups about number of macrophage cells and capsule thickness, but there are significant differences between experimental group and control and sham groups. Detailed observations showed significant increase in the volume of monocyte, neutrophile and eosinophile, in response to QPPE. Statistical results about the volume of lymphocyte and basophile cells showed no changes in experimental group in compared with control and sham groups.

Discussion

Quinazolinones are heterocyclic, water insoluble and lipophilic compounds, with various pharmacological characteristics; (antimicrobial, antifungal, antiswelling, Parkinson and etc.) (David et al., 2005, James et al., 1990, Pines et al., 2000, Buyuktimkin et al., 2006, Jatav et al., 2006, Yesilada et al., 2004, Ouyang et al., 2006 and Baek et al., 2004). They are more efficient than other chemicals in inhibiting HIV and cancer (Corbett et Mann et al., 2006). Xia et al. (2001) displayed quinazolinone as a potent inhibitor of tubulin polymerization. They enter

circulatory system and passes through placental barrier (Perretti and Zilletti, 1969).

With due attention to results of earlier researches and to observe morphological abnormalities, skeletal malformation (Shams lahijani et al., 2006, Shams lahijani and Aounegh, 2007) and damage in liver, intestine and kidney (Etemad and Shams lahijani, 2007, Fadavi and Shams lahijani, 2007 and Rajabi and Shams lahijani, 2007), brain, heart and stomach [in progress], we investigate effects of quinazolinones on the spleen development and immunucompetent cells in mouse Balb/C.

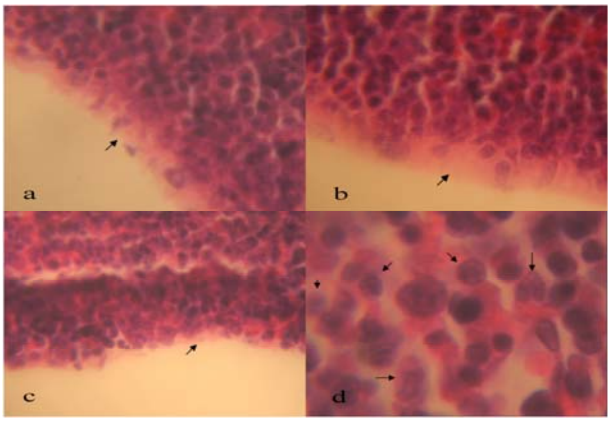


Figure.2: a; Normal spleen capsule of control group that treated with 10 ml/kg body weight of distilled water (H&E, 400X) b; . Normal spleen capsule of sham group that treated with 10 ml/kg body weight of methyl cellulose %0.05 (H&E, 400X). c; Increase of spleen capsule of experimental group that received with 100 mg/kg body weight of QPPE (H&E, 400X). d; Cross section of spleen of treated group with 100 mg/kg body weight of QPPE, having large number of macrophage cells (arrows, H&E, 1000X).

Results of present study showed that treatment with quinazolinones induce splenomegaly and increase capsule thickness. Measurements of immune-related hematological parameters in mouse blood show that QEPE causes monocytosis, neutrophilia and eosinophilia.Splenomegaly is often seen in hypersplinism, increase of phagocitic cells (especially macrophage cells) and external articles in billroth's cords, patients with hepatic cirrhosis or portal hypertension and damage in liver and heart (Sama and Moro, 1993, Paula et al., 1998, Bordmann et al., 1997, Wanless and Bernier, 1983 and Radovan, 1987). Most causes of splenomegaly in this research including: 1-Damage in liver, heart and other organs. 2- Increase of macrophage and immunocompetent cells. Monocytes circulate in the blood stream and differentiate into specific tissue macrophages which are actively phagocytic cells capable of ingesting and digesting exogenous such as the whole bacterial cells, virus particles, and injured or dead host cells (Richard et al., 1997).Treatments of mice with these components lead to damages in liver, intestine, kidney, heart, stomach and brain. These damages can cause increase in the number of monocyte and macrophage cells. Some experiment have shown that, hyperlipidemia increase the number of macrophage cells in spleen. These macrophage cells interfere in removal of lipid from circulatory system. QEPE causes the increase and fullness of lipid in hepatic hepatocytes (Rajabi and Shams lahijani, 2007). It appears that QEPE disorders the metabolism of lipids and create hyperlipidemia. Also, these tow compound can cause neurophilia and eosinophilia. It is possible that, QEPE can increases migration of eosinophils and neutrophils from bone marrow to the blood stream. Eosinophils, like neutrophils, are motile, phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is less important than that of neutrophils, and it is thought that their major role consists in defense against parasitic organisms (Richard et al., 1997). However, neutrophils are the first cells that arrive at a site of inflammation during response to many types of infection. Before investigations had shown that these two compounds can cause necrosis in some organs such as; intestine, kidney (Etemad and Shams lahijani, 2007 and Fadavi and Shams lahijani, 2007) heart and stomach [in progress]. While

creation of necrosis increase white blood cells, macrophage cells and defensive factors.

Increase of splenic capsule thickness is often seen in patients with hepatic cirrhosis or portal hypertension from other causes and in liver and heart (Wanless and Bernier, 1983). Other causes of splenic capsular thickening are increase of matrix contents and increase of blood cells and macrophage cells (Radovan, 1987). In this study it is probable that most cause of splenic capsular thickening are caused by damages in liver and heart, and increase of macrophage cells.

We concluded that Treatments of mice with these components lead to damages in liver, intestine, kidney, heart, stomach and brain. These damages can cause increase in the number of immonucompetent and macrophage cells. Some studies have shown that damage in liver and heart lead to increase of capsule thickness. Indeed this factor causes increase in weight and leads to splenomegaly. Therefor, QEPE can not be an appropriate candidate for drugs development. Maybe QEPE in the lower dose can be an appropriate candidate for increase of immuno system resistance.

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مطالعه اثرات ایمونوپاتولوژیک 4(3H)-quinazolinone-2-ethyl-2-phenyl ethyl (QEPE) در نوزاد موش سوری

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چکیده

کینازولینون‌ها ترکیباتی هتروسیکلیک با فعالیت‌های فارماکولوژیک متنوع (ضد باکتریایی، ضد قارچ، درمان تومور و ایدز) هستند. هدف از تحقیق حاضر، بررسی اثر یک ترکیب جدید از کینازولینون ها به نام،4(3H) quinazlonones-2-ethyl-2-phenyl ethyl (QEPE) روی سلول‌های صلاحیت دار سیستم ایمنی و طحال نوزادان موش باردار نژاد Balb/C می باشد. موش‌های باردار به سه گروه (n=10)، کنترل، شم و آزمایشی تقسیم و در روز ۸ تا ۱۵ بارداری به ترتیب آب مقطر، متیل سلولز ۵٪ (حلال کینازولینون ها) و ۱۰۰ میلی گرم به ازای هر کیلو گرم وزن بدن از QEPE دریافت کردند. نمونه‌های خون از قلب نوزادان ۵ روزه و همچنین طحال آنها برای مطالعه برداشته شد. بررسی آماری نشان داد که افزایش معنی داری در وزن طحال، ضخامت کپسول و تعداد سلولهای ماکروفاژ طحال (P<0.05) گروه آزمایشی نسبت به گروه‌های کنترل و شم وجود دارد. نتایج آنالیز خون نشان داد تعداد سلول‌های مونوسیت، نوتروفیل و ائوزینوفیل گروه آزمایشی نسبت به گروه‌های کنترل و شم افزایش معنی داری یافته است (P<0.05) ، اما تعداد سلول‌های لنفوسیت و بازوفیل در بین سه گروه مشابه است و اختلاف معنی داری ندارند. آ سیب‌های بافتی حاصل از QEPE در این دوز می تواند دلیلی بر ایجاد این نتایج باشد. ما نتیجه گرفتیم که ترکیب QEPE در این دوز نمی تواند کاندید مناسبی برای ساخت داروهای جدید باشد و همچنین نمی توان از آن در دوران بارداری استفاده کرد. شاید این ترکیب در دوزهای پایین تر بتواند کاندید مناسبی برای افزایش مقاومت سیستم ایمنی باشد.

واژه‌های کلیدی: کینازولینون، سلول‌های صلاحیت دار سیستم ایمنی، طحال، موش نژاد Balb/C.